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A flavonoid survey of *Fraxinus* (Oleaceae) in eastern Asia, and the overlooked species *Fraxinus hopeiensis* T. Tang in northern China

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ABSTRACT

From a survey of 14 flavonoids of 19 Asian taxa of Fraxinus, flavonols were present in most taxa of sects. *Ornus*, *Fraxinus*, and *Sciadanthus*, while flavones were only detected in *Fraxinus lanuginosa* in Japan and *Fraxinus hopeiensis* in China of sect. *Ornus* and *Fraxinus chiisanensis* in Korea (as *incertae sedia sensu* Wallander). The present data failed to clearly distinguish many taxa within the genus because of the extreme monotony of flavonoid content. However, the dichotomy on a smaller scale was observed in *Fraxinus*, where this chemical approach was useful in the determination of natural groupings of the overlooked taxon *F. hopeiensis* in China, *F. lanuginosa* in Japan, and *F. chiisanensis* in Korea. The gain of O-flavone appears to be a significant evolutionary step; however, the sporadic occurrence of O-flavone in some species is the result of chemical advancement. The present study demonstrated that flavonoid chemistry may indeed be as variable as the morphological features, with no significant flavonoid differences observed at the species level. However, *F. hopeiensis*, found in the mountain regions of Hebei in northern China, has petals like *Fraxinus sieboldiana* and *F. lanuginosa* and is clearly different from other related taxa, including *Fraxinus rhynchophylla* and *Fraxinus chinensis*. This study shows that *F. hopeiensis* is morphologically, chemically, and geographically distinct and should be treated as a distinct species.

1. Introduction

The genus *Fraxinus* of Oleaceae comprises approximately 70 woody species, which are widely distributed in Europe, Eastern Asia, and North and Central America (Willis, 1985; Lingelsheim, 1920; Chang et al., 1992; Wei and Green, 1996; Wallander, 2008). The major classifications by Lingelsheim (1920), Chang et al. (1992), Wei and Green (1996), and Wallander (2008) were discordant in terms of classification. The two subgeneric groupings, *Ornus* (Boehm.) Peterm. and *Fraxinus* [=*Fraxinaster* (DC.) V. Vassil., *sensu* Lingelsheim (1920) and Vassiljev (1952)] by Nikolaev (1981) and Chang et al. (1992) sharply contrasted with the six sections [*Fraxinus*, *Dipetalae* (Lingelsh.) E. Nikolaev., *Ornus* (Boehm.) DC., *Melioides* (Endl.) Pfeiff., *Sciadanthus* (Coss. Et Dur.) Lingelsh., and *Pauciflorae* (Lingelsh.) E. Wallander] proposed by Wallander (2008), based on her DNA studies and exomorphological characters. Wallander's classification is the currently most frequently accepted treatment.

Among the six sections sensu Wallander (2008), sects. *Ornus*, *Sciadanthus*, and *Fraxinus* are mainly restricted to Eastern Asia. Sect. *Ornus* contains more than nine recognized species growing in China and three additional known species from

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either Japan or Korea, while only two species are grouped in sect. *Fraxinus* in China. Given the distribution patterns of these sections, the center of diversification is clearly situated in China or North and Central America (Chang et al., 1992; Wei and Green, 1996; Wallander, 2008).

In a chemical appraisal of family Oleaceae by foliar flavonoids, Harborne and Green (1980) presented an expanded discussion and detailed documentation in which the flavonoids chemical dichotomy provided strong evidence in support of phylogeny and evolution. Harborne and Green (1980) showed that the possession of flavones of *F. chinensis* var. *rhynchophylla* (Hance) Hemsl. (=*Fraxinus rhynchophylla*) and *Fraxinus americana* L. differed greatly in their flavonoid profile from other related taxa, which possessed flavonols. However, our preliminary data were not in accordance with the report of Harborne and Green (1980) on *F. chinensis* var. *rhynchophylla* because of the presence of flavonols.

Recent work by Min et al. (2001) showed the taxonomic utility of flavonoids in the species delimitation of *Fraxinus chiisanensis* Nakai in Korea. A survey of the foliar flavonoids of a limited number of species in eastern Asia showed that unique flavones with a few flavonols were detected in *F. chiisanensis*, while only flavonols were present in other examined taxa (Min et al., 2001). Several DNA phylogeny studies (Noh et al., 1999; Wallander, 2008) indicated that *F. chiisanensis* was not a hybrid between *Fraxinus mandshurica* Rupr. and *F. rhynchophylla*, unlike the conclusions of previous literatures (Chung, 1957; Lee, 1980), but rather an endemic taxon which was distributed in southwestern Korea. Morphologically, *F. chiisanensis* is clearly distinguished from either *F. rhynchophylla* or *F. mandshurica* due to the presence of panicle from leafless lateral buds of the previous year, apetalous flowers, persistent calyx, and brownish buds (Min et al., 2001).

Relatively little systematic work on the flavonoids survey of Asian ash has been conducted; however, our works (Min et al., 2001; Chang et al., 2002; Kang et al., 2002) have shown that flavonoid data was quite effective in a recent study of *Fraxinus*. The next logical step was to examine all Chinese taxa to determine if the flavonoid profiles were more effective for confirmation of a certain identity at the species level.

Thus, it was considered desirable to determine: 1) if any of the taxa are characterized by unusual flavonoid compounds; 2) the extent of variation in the flavonoid chemistry and to its correlation with various interspecific variations.

2. Materials and methods

Field collections representing 19 taxa (including *F. szaboana* Lingelsh.) of *Fraxinus* were sampled in Korea, Japan, and China. Herbarium numbers and country names for each of the species examined are given in Table 1. Additionally, small leaf fragments of individuals obtained from PE, KUN, TI, SZ, CDBI, MAK, and KYO herbaria sheets were surveyed.

According to Chang et al. (1992), *F. chinensis* is distributed from China to Vietnam, including Korea, while *F. rhynchophylla* is reported from Northeastern China, far eastern Russia, Korea, and Japan to the eastern part of China (Gansu, Hebei, Henan, Shaanxi, Shandong, and Shanxi). Alternatively, Fu (1995) clearly indicates that only *F. chinensis* in Northeastern China is known to be strictly distributed in Liaoning, but *F. rhynchophylla* is widely found in Heilongjiang, Jilin, and Liaoning. Therefore, samples referred to as *F. chinensis* are from the southern parts of China, excluding Heilongjiang and Jilin, and var. *F. rhynchophylla* is from northeastern China (Heilongjiang, Jilin, and Liaoning) and Korea.

The extraction of flavonoids from leaves and flowers followed the methods of Mabry et al. (1970), Chang and Giannasi (1991), and Giannasi (1975). The flavonoids survey employed two-dimensional paper chromatography using Whatmann 3 mm paper. TBA (tertiary-butanol:acetic acid:water, 3:1:1, v/v) was used as the first extraction solvent, and 15% HOAc (acetic acid:water, 15:85, v/v) was used as the second solvent. Flavonoid profiles were viewed under UV light, and the resulting colors were recorded before and after fuming with ammonia vapor. Compounds were identified using standard UV–visible spectroscopy (Giannasi, 1975; Mabry et al., 1970; Markham, 1982). Rf values of the purified compounds in TBA and HOAc were recorded. Compounds thought to be the same in different taxa were co-chromatographed for verification. Spectral and Rf data were compared with published data (Mabry et al., 1970). Flavonoid glycoside sugars were identified using trifluoroacetic acid hydrolysis and one-dimensional co-chromatography with standard sugars, using ethyl acetate:pyridine:water (5:1.6:1, v/v) as the solvent. The sugars were visualized by spraying with *p*-anisidine hydrochloride (Pridham, 1956), followed by heating (100 °C, 10 min). Several compounds were compared directly with compounds identified from previous studies (Chang and Giannasi, 1991).

The extracts were also analyzed by an Agilent 1100 HPLC and a Bruker HCT 3000 mass spectrometer equipped with an ESI interface. The ESI parameters were as follows: capillary voltage, 4.5 kV; dry temperature, 365 °C; nebulizer gas, 50 psi; and dry gas, 9 L/min. The mass spectrometer was operated in positive or negative ion modes scanning from m/z 100 to 800. An Xterra MSC-18 column (3.0 × 150 mm, 3.5 μm, Waters Co., Milford, MA) was used at a column temperature of 30 °C. The mobile phase was a binary elution of (A) 1% aqueous acetic acid solution and (B) 1% acetic acid in acetonitrile under the following gradient conditions: 0–5 min isocratic at 90% of A, 5–15 min linear gradient from 90% to 50% of A, 15–20 min linear gradient from 50% to 0% of A, and 20–30 min isocratic at 100% B. The sample injection volume was 5 mL, and the flow rate was 0.5 mL/min. Flavonoids in the extracts were identified by LC–MS in addition to the paper chromatography. MS and MS/MS spectral analyses were performed as in a previous report (Kim and Park, 2009).

3. Results

The results of the flavonoids survey are presented in Table 2, where the distribution of 14 flavonoids among 19 taxa in Asia is shown. A few remained unidentified due to their occurrence in trace amounts too low for successful analysis.

Table 1
Origin and accession number for specimens used for flavonoids surveys. All voucher specimens collected by authors were deposited at the herbarium (T. B. Lee Herbarium, SNUA) of The Arboretum, Seoul National University, and each acronym represented herbaria as follows; CDBI: Herbarium Chengdu Institute of Biology [Chengdu Institute of Biology], KYO: Herbarium Botany Department Graduate School of Science Kyoto University [Kyoto University], KUN: Herbarium Kunming Institute of Botany, Chinese Academy of Sciences [Kunming Institute of Botany, Chinese Academy of Sciences], MAK: Makino Herbarium Tokyo Metropolitan University [Tokyo Metropolitan University], PE: Chinese National Herbarium Institute of Botany, Chinese Academy of Sciences [Institute of Botany, Chinese Academy of Sciences], SZ: Herbarium Biological Department Laboratory of Systematic Botany Sichuan University [Sichuan University].

Taxa	Origin and accession number
F. baroniana Diels	China: KUN27532, PE174258, PE6751, PE274238
F. bungeana DC.	China: PE6699, PE6704, PE6705, PE6707, PE6708, PE6711, PE7427, PE7605, PE7650, PE43435, PE60844, PE252596, PE351067, PE313183, PE315015, PE569359, PE562488
F. chinensis Roxb.	China: CDBI113791, CDBI113793, CDBI 113794, CDBI113813, CDBI 113820, KUN27522, KUN27581, KUN27583, KUN27588, KUN27589, KUN27590, KUN27591, KUN27592, KUN27595, KUN27597, KUN27598, KUN27600, KUN27607, KUN27610, KUN27628, KUN27641, KUN27643, KUN27648, KUN27650, KUN27651, KUN28014, KUN28017, PE223, PE2915, PE21733, PE71101, PE75899, PE336512, PE709918, PE2742532, PE6290717, SZ75867, SZ75872, SZ75874, SZ75876, SZ75878, SZ75879, SZ75895, SZ75896, SZ280532, SZ280554, SZ280557, SZ280559, SZ280561, SZ280567, SZ280570, SZ281818,
F. chiisanensis Nakai	Korea: SNUA57972, SNUA57973, SNUA57974, SNUA57975, SNUA57976, SNUA57977, SNUA57978, SNUA57979, SNUA57981, SNUA57982, SNUA57983, SNUA57984, SNUA57985, SNUA60237, SNUA60238, SNUA60239, SNUA60240, SNUA60247, SNUA60248, SNUA60250, SNUA60251, SNUA60252, SNUA60253, SNUA60245, SNUA60256, SNUA60257, SNUA60258, SNUA61644
F. floribunda Wall. ex Roxb.	China: PE298022, PE3504, PE42634, PE903362, PE1311797, MAK203034, P.C.Tsoong3376(PE) (as *F. stylosa* Lingelsh), PE274240 (as *F. stylosa*), PE560294 (as *F. stylosa*), PE229 (as *F. odonthocalyx* Hand.-Mazz.), PE313016 (as *F. odonthocalyx*) PE720932 (as *F. odonthocalyx*), PE722761 (as *F. odonthocalyx*), PE316010 (as *F. odonthocalyx*), Q.G.He *s.n.* June 29,1979(PE) (as *F. odonthocalyx*), PE770005 (as *F. insularis* Hemsl.), PE770017(as *F. insularis*), PE1277677 (as *F. insularis*), Japan: MAK22347 (as *F. insularis*), MAK180870 (as *F. insularis*), MAK328900 (as *F. insularis*), KYO410 (as *F. insularis*)
F. griffithii C. B. Clarke	China: PE586947, PE1255306, PE1647148
F. hopeiensis T. Tang	China: PE968, PE1897, PE3316, PE378536, PE598232, PE666294, PE683617, PE683627, PE693202, PE789894, PE875479, PE13212693, PE1545477, PE2212694, PE2212701, PE2212702, PE2212704, PE2212728, PE2212736, PE2212738, PE2212766, KUN27657, KUN27659, KUN27656, KUN27660
F. hupehensis Ch'ű, Shang & Su	China: K. Kimura *s.n.* Apr.28.1939(PE)
F. lanuginosa Koidz.	Japan: MAK6941, MAK170320, MAK202053,MAK302875, MAK 302876, PE1200428, PE1200429,
F. longicuspis Siebold & Zucc	Japan: MAK 93945, MAK 135459, MAK 150858, MAK 170334, MAK170338, MAK 180858
F. malacophylla Hemsl.	China: PE11617, PE13471, PE37765, PE188015, PE340313, PE346311, PE588415, PE311765
F. mandshurica Rupr.	Korea: SNUA66515, SNUA66533, SUNA66534, SNUA66535, SNUA66536, SNUA66537, SNUA66538, SNUA79580
F. paxiana Lingelsh.	China: PE8479, PE346287, PE341687, PE572835
F. platypoda Oliv.	China: PE627916, PE1017548, PE1553224, KUN27750
F. rhynchophylla Hance	Korea: SNUA54723, SNUA57969, SNUA60233, SNUA60238, SUNA61616, SNUA61617, SNUA61629, SNUA61645, SNUA66495, SNUA66543, SNUA79621, SNUA79628, SUNA82561,SNUA82562 China: PE1153, PE351068, PE681435, PE739899, PE753407, KUN27661, KUN27663, KUN27674, KUN28000 Japan: MAK170313 (as *F. japonica*), MAK170314 (as *F. japonica*), MAK199803 (as *F. japonica*) China: PE259271 (as *F. szaboana*), PE211 (as *F. szaboana*), PE212 (as *F. szaboana*), PE2603 (as *F. szaboana*), PE6722 (as *F. szaboana*), PE560296 (as *F. szaboana*), PE22 (as *F. szaboana*), PE1107546 (as *F. szaboana*), PE43154 (as *F. szaboana*), PE6718 (as *F. szaboana*), PE10080 (as *F. szaboana*), PE13720 (as *F. szaboana*), PE40942 (as *F. szaboana*), PE274243 (as *F. szaboana*), PE423812 (as *F. szaboana*), PE1488701 (as *F. szaboana*), PE2212848 (as *F. szaboana*), PE2212850 (as *F. szaboana*), PE2212857 (as *F. szaboana*), PE2212854 (as *F. szaboana*), PE2212944 (as *F. szaboana*), PE11047 (as *F. szaboana*), PE423812 (as *F. szaboana*), PE395569 (as *F. szaboana*), PE2214716 (as *F. szaboana*), PE410134 (as *F. szaboana*), PE274242 (as *F. szaboana*), PE6718 (as *F. szaboana*), PE1017566 (as *F. szaboana*), PE2212855 (as *F. szaboana*), PE2212856 (as *F. szaboana*), PE2212851 (as *F. szaboana*), PE2212849 (as *F. szaboana*), PE2214716 (as *F. szaboana*), PE274251 (as *F. szaboana*), PE2212944 (as *F. szaboana*)
F. sieboldiana Blume	Korea: SNUA57987, SUNA60241, SUNA61633, SNUA61634, SNUA61635, SNUA66529, SUNA66531, SUNA66545, SNUA66546, SNUA66548, SNUA79577, SNUA79581, SUNA79590, SUNA79620
F. augustifolia Vahl	China: PE1118205 (as *F. sogdiana* Bunge)
F. spaethiana Lingelsh.	Japan: TI16679, TI1317683
F. xanthoxyloides (G. Don) DC.	China: PE00195

From the survey of 14 flavonoids, O-flavones and flavonols were characterized. The identities of the flavonoids were: quercetin 3-O-galactoside, quercetin 3-O-glucoside, quercetin 3-O-rhamnoglucoside, quercetin 3-O-arabinoside, quercetin 3-O-glycoside, kaempferol 3-O-galactoside, kaempferol 3-O-glucoside, kaempferol 3-O-glycoside, luteolin 7-O-rhamnoglucoside, luteolin 7-O-glucoside, apigenin 7-O-glycoside (sugar unknown1), apigenin 7-O-rhamnoglucoside, apigenin 7-O-glycoside (sugar unknown2), and apigenin 7-O-glucoside.

The results suggested that flavonols were more common in the Asian *Fraxinus* taxa, but two basic flavonoid types were also observed, i.e., flavonols and flavones with a limited number of flavonols. Within the flavonols, quercetin and kaempferol were the most common parent aglycones and were present in most of the analyzed taxa of sect. *Ornus* and sect. *Fraxinus*. As far as substitution trends are concerned, there was a strong tendency toward the formation of flavonol 3-O-glycosides derivatives. A

Table 2
Flavonoids distribution in *Fraxinus* taxa considered. Compounds 1–8 Flavonol glycosides; 9–14 flavone glycosides. (x) means compounds detected in less than 50% of surveyed individuals. Flavonoids data about North American taxa of sect. *Melioides* presented here were adapted from Chang et al. (2002).

Species	Flavonol group								Flavone group					
	Quercetin					Kaempferol			Luteolin		Apigenin			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sect. *Ornus*														
F. sieboldiana	x		x	(×)			x	(×)						
F. lanuginosa	x		x		x	x				x		x		x
F. floribunda	x			x			x							
F. rhynchophylla	x		x	x			x							
F. baroniana	x			x			x							
F. chinensis	x		x				x							
F. longicuspis	x		x				x							
F. bungeana	x		(×)				x							
F. hopeiensis			x	x						x	x	x		
F. griffithi	x		x				(×)							
F. paxiana	x		x				x							
F. malacophylla	x		x				x							
F. szaboana		x	x		x	x								
Sect. *Sciadanthus*														
F. hupehensis			x					x						
F. xanthoxyloides		x	x		x	x								
Sect. *Fraxinus*														
F. mandshurica		x	x		x	x								
F. spaethiana		x	x		x	x		x						
F. platypoda		x			x	x								
F. augustifolia		x	x			x								
Sect. *Melioides*														
F. velutina		x	x			x								
F. profunda		x	x						x	x	x	x		x
F. papillosa			x			x			x	x			x	x
F. americana			x			x			x	x	x	x		x
F. caroliniana		x	x						x	x	x	x		x
F. pennsylvanica		x	x			x			x	x	x	x		x
F. chiisanensis					x				x	x	x	x	x	x

1, quercetin 3-O-galactoside; 2, quercetin 3-O-glucoside; 3, quercetin 3-O-rhamnoglucoside; 4, quercetin 3-O-glycoside; 5, quercetin 3-O-arabinoside; 6, kaempferol 3-O-galactoside; 7, kaempferol 3-O-glucoside; 8, kaempferol 3-O-glycoside; 9, luteolin 7-O-rhamnoglucoside; 10, luteolin 7-O-glucoside; 11, apigenin 7-O-glycoside(sugar unknown); 12, apigenin 7-O-rhamnoglucoside; 13, apigenin 7-O-glycoside(sugar unknown); 14, apigenin 7-O-glucoside.

large survey of the genus showed uniform flavonols, while species delimitation within sect. *Ornus* in eastern Asia was limited. Alternatively, flavones, primarily luteolin 7-O-glucoside and apigenin 7-O-rhamnoglucoside, were detected in both *Fraxinus lanuginosa* in Japan and *Fraxinus hopeiensis* in China, belonging to sect. *Ornus*.

Some taxa of *Fraxinus* in eastern Asia, however, could be further divided into two groups based on the different combinations of quercetin and kaempferol 3-O-glycoside(s) compounds: *Fraxinus griffithii* C. B. Clarke, *Fraxinus floribunda* Wall. ex Roxb. including *Fraxinus insularis* Hemsl., *Fraxinus odontocalyx* Hand.-Mazz., and *Fraxinus stylosa* Lingelsh., *Fraxinus paxiana* Lingelsh., *Fraxinus baroniana* Diels, *F. rhynchophylla*, and *F. chinensis* belonged to sect. *Ornus*, while *F. mandschurica*, *Fraxinus platypoda* Oliv., *Fraxinus augustifolia Vahl (as Fraxinus sogdiana Bunge)* and *Fraxinus xanthoxyloides* (G. Don) DC. were included in sects. *Fraxinus* and *Sciadanthus*.

4. Discussion

There was a remarkable overall similarity in the flavonoid profiles within the sections. Because of the extreme monotony of the flavonoid content, far less information was obtained than was hoped. Most species could not be discriminated by this approach. However, this study found that *Fraxinus* in eastern Asia could be differentiated by the distribution of the two biochemically different groups of flavonoids, mainly flavonols and flavones with a few flavonols. The former group was characterized by higher quercetin and kaempferol 3-O-glycosides diversity, while the derived group of *F. lanuginosa* and *F. hopeiensis* including *F. chiisanensis* was generally characterized by many flavones and reduced flavonol profiles.

4.1. Sects. Fraxinus and Sciadanthus

Within the flavonols group, two sections, *Fraxinus* and *Sciadanthus*, were distinct from sect. *Ornus* in the possession of quercetin 3-O-glycoside(s) and kaempferol 3-O-glycoside(s). This taxonomic division within genus *Fraxinus* was well supported not only by these flavonoid results, but also by ITS sequences (Jeandroz et al., 1997; Wallander, 2008).

Due to the paraphyletic relationship of *F. chiisanensis* with taxa of sect. *Fraxinus* and polygamous type flowers, Wallander (2008) classified *F. chiisanensis* as *incertae sedis*. A survey of the foliar flavonoids showed, however, that flavones with flavonols were also detected in four North American ash taxa of sect. *Melioides* (Min et al., 2001; Chang et al., 2002) containing flavones, and that they were chemically more closely related to *F. chiisanensis*.

With respect to flavonoid evolution (Harborne, 1977; Gornall and Bohm, 1978; Giannasi, 1978, 1988), sects. *Ornus*, *Fraxinus*, and *Sciadanthus* were considered to be a primitive group due to the absence of flavones. Evolutionary advancement probably accompanied a loss of some flavonols and a gain of O-flavone syntheses, resulting in sect. *Melioides*. The previous ITS trees proposed by Wallander (2008) and Jeandroz et al. (1997), however, indicated sect. *Melioides* appeared paraphyletic with the other three sections and did not support the evolutionary advancement of sect. *Melioides*. These flavonoid evolutionary trends simply reflected sectional parallelisms in flavonoid evolution within the genus.

4.2. Sect. Ornus

Sect. *Ornus* is composed of two subsections: *Ornus* (=*Euornus*) and *Ornaster sunsu* Lingelsheim (1920). Subsect. *Ornus* comprises hermaphroditic or androdioecious flowers with four free petals, while subsect. *Ornaster* is characterized by androdioecious or dioecious apetalous flowers. This taxonomic treatment was not supported by Wallander's (2008) study due to the paraphyletic relationship between the two subsections. Morphologically, *F. hopeiensis* has petals like those of *Fraxinus sieboldiana* and *F. lanuginosa* (=subsect. *Ornus*) and is clearly different from subsect. *Ornaster sensu* Lingelsheim. Flavonoid profiles of *F. sieboldiana* and other taxa of sect. *Ornus* showed only the presence of flavonols, while *F. lanuginosa* possessed a profile nearly identical to that of *F. hopeiensis*. Flavonoid analyses indicated that the group may be characterized according to the presence of petals with flavones, although *F. sieboldiana* had only flavonols. Nakaike (1972) indicated that *F. lanuginosa* is quite widespread, ranging from Hokkaido to Kyushu and eastward through southern Korea. Flavonoid profiles of collections throughout much of the range in southern Korea are quite uniform, consisting of flavonol glycosides. Thus, *F. lanuginosa* is endemic to Japan and is closely related to *F. sieboldiana* in China, Korea, and Japan but differs in its pubescent bud scale.

The evolutionary gain of O-flavone appears to be a significant advancement (Harborne, 1977); however, the sporadic occurrence of O-flavone in some species within the genus may seemingly be the result of chemical advancement. It is suggested that parallel and/or radiate modes of chemical evolution should be applied within this genus.

4.3. An overlooked species

F. hopeiensis bears a striking but superficial resemblance to *F. rhynchophylla* as herbarium material. Tang (1931) originally characterized *F. hopeiensis* from Hebei as resembling forms of either *F. longicuspis* in Japan or *F. rhynchophylla* in China but emphasized distinguishing characters such as leaflet shapes, serration patterns, and petalous flowers (Fig. 1). On the other hand, Chang et al. (1992) differed from Tang (1931) by reducing *F. hopeiensis* to a synonym of *F. rhynchophylla* in the Chinese version of Flora of China because he believed that petals in *F. hopeiensis* were an abnormal type of the apetalous flowers in *F. rhynchophylla*. The current flavonoid survey of *F. hopeiensis* was quite distinct in comparison to that of sect. *Ornus* due to the presence of flavones. According to earlier investigations on flavonoids of Oleaceae by Harborne and Green (1980), samples from flavones in *F. rhynchophylla* suggest that the group should be classified as *F. hopeiensis*.

F. hopeiensis in Hebei exhibites morphological similarity compared with the related taxa, *F. bungeana* A. de Candolle in Northern China and *F. floribunda* Wallich in Southern China. *F. bungeana* is superficially similar to the form of *F. hopeiensis* since it has a similar flowering season and is also found in Hebei, as well as other northern provinces of China. However, our observation revealed evident differences between *F. bungeana* and *F. hopeiensis* with respect to habit (shrub vs. tree), leaflet size (2–5 × 1.5–3 cm vs. 6–9 × 4–6 cm), petiolule length (2–15 mm vs. 10–15 mm), leaflet blade (sharply serrate vs. crenate

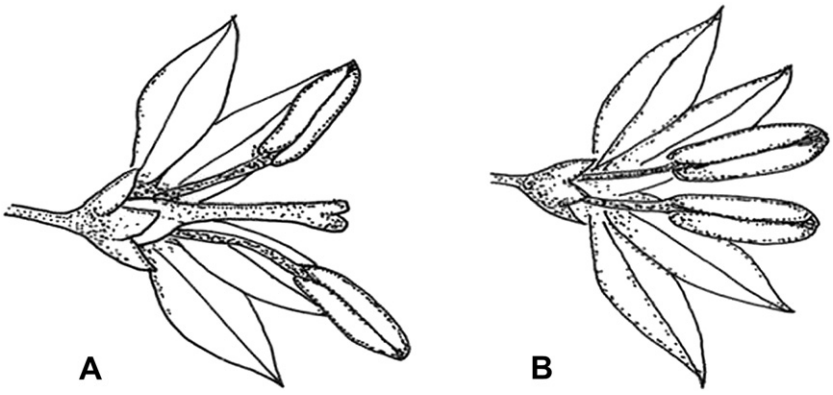

Fig. 1. Floral structures of *F. hopeiensis* (A: bisexual flower B: male flower).

Fig. 2. Distribution of *F. hopeiensis* in Hebei of China. Circle marks are marked based on all herbarium specimens (PE) examined.

distantly), and terminal bud shape (conical vs. ovoid). This study also shows that *F. floribunda* differs from *F. hopeiensis* due to a generally greater leaflet number [(5)7–9], a densely tomentose terminal bud, and an early flowering season (Feb.–April). *F. floribunda*, which has never been found in Hebei, is known to be restricted to southern parts of China, such as Guangdong, Guangxi, Guizhou, Xizang, Yunnan, and Zhejiang.

Our flavonoid survey agrees with Tang's concept concerning the status of this taxon and it is thus treated as a distinct species. Indeed, the presence of petals and the late flowering season (late May) of *F. hopeiensis* easily distinguished it from those of *F. rhynchophylla*. The presence of petals in *F. hopeiensis* is distantly related to either Japanese endemic *F. lanuginosa* or *F. sieboldiana* in Korea and Japan, but the current flavonoid survey provides some evidence to link *F. hopeiensis* to *F. lanuginosa* rather than to *F. sieboldiana*.

Fraxinus hopeiensis T. Tang, Bull. Fan Mem. Inst. Biol. 2: 101, 1931.

TYPE: China Prov. Hebei, Mt. Miao-feng-shan, near Peiping (=Beijing). H. Chow no. 40239 (holotype, PE!).

DISTRIBUTION: endemic to Hebei of Northern China (Fig. 2); distributed very locally in deciduous forests at altitudes of 700–800 m.

In the Chinese version of Flora of China, Chang et al. (1992) designated *F. szaboana*, *F. chinensis*, and *F. rhynchophylla* as species, while Chang et al. (1996), in the English edition of Flora of China, reduced *F. rhynchophylla* to a subspecies of *F. chinensis* and *F. szaboana* to a synonym of *F. chinensis* subsp. *chinensis*. The species delimitation of *F. szaboana* from Northeast China was difficult because of the overall high degree of morphological similarity with *F. rhynchophylla* and/or *F. chinensis*. Our chemical analysis of *F. szaboana* showed a relationship to *F. mandshurica*, as opposed to *F. chinensis* or *F. rhynchophylla*. While the specimens differ from *F. rhynchophylla* in terms of flavonoids, the leaf blades and terminal bud pubescence are more or less similar to those of *F. rhynchophylla*. It seems premature to classify it formally based on this study.

Due to a very short flowering season of ash taxa, the floral morphology of *Fraxinus* is often a difficult character to detect. Although Wallander (2008) helped to clarify the patterns in the evolutionary history of the reproductive traits in the entire genus, this current study pointed out the critical nature of ash flowers in identification and species delimitation. Often, specimens lacking flowers are very common in herbaria and are virtually difficult to identify species unless one is very familiar with the taxa involved and has a first-hand appreciation of the variation within a particular taxon. Therefore, on the basis of herbarium specimens alone, it was not possible to conclusively demonstrate whether cryptic species were actually present.

In conclusion, the dichotomy on a smaller scale was observed in *Fraxinus*, where this chemical approach was useful for the determination of natural groupings of the overlooked species *F. hopeiensis* in China, *F. lanuginosa* in Japan, and *F. chiisanensis* in Korea.

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